

# Polymerase Chain Reaction (PCR)

## Materials

- 10 mM dNTP mix
- 10  $\mu$ M Forward primer
- 10  $\mu$ M Reverse primer
- DNA template (10 pg to 1  $\mu$ g)
- Water, nuclease free
- Thin walled PCR tubes

For High Fidelity enzyme amplification:

- High Fidelity Enzyme Mix (5U/ $\mu$ L)
- 10X High Fidelity PCR Buffer with 15 mM  $MgCl_2$

For Taq DNA Polymerase amplification:

- 10X Taq Buffer with  $(NH_4)_2SO_4$
- 25 mM  $MgCl_2$
- Taq Polymerase (5U/ $\mu$ L)

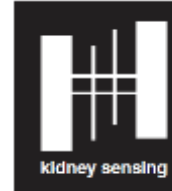
## Apparatus

- Thermocycler (Applied Biosystems)

## Method

- After thawing and vortexing the solutions (except the enzyme solution), add the components in a sterile PCR tube in this following order and keep it on ice.

10X High Fidelity PCR Buffer with 15 mM $MgCl_2$	5 $\mu$ L	Taq Polymerase (5U/ $\mu$ L)	5 $\mu$ L
10 mM dNTP mix	1 $\mu$ L	10 mM dNTP mix	1 $\mu$ L
10 $\mu$ M Forward primer	1 $\mu$ L	10 $\mu$ M Forward primer	1 $\mu$ L
10 $\mu$ M Reverse primer	1 $\mu$ L	10 $\mu$ M Reverse primer	1 $\mu$ L
DNA template Genomic DNA	10 pg- 1 $\mu$ g	25 mM $MgCl_2$	4 $\mu$ L
High Fidelity Enzyme Mix	0,5 $\mu$ L (2.5 U)	DNA template Plasmid DNA	10 pg- 1 $\mu$ g
Water, nuclease free	to 50 $\mu$ L	Taq DNA Polymerase	0.5 (2.5U)
		Water, nuclease free	to 50 $\mu$ L



2. Spin the PCR tubes to collect all drops and immediately place the PCR tubes in the thermocycler, start the PCR reaction.
3. Thermal cycling protocol:

Obs: Because the primers used have more than 12 pb (including restriction sites) that don't anneal with the template in the first PCR steps, we decided to add 10 cycles with a lower temperature to favor the best annealing.

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94-95	3 min	1
Denaturation	94-95	30 s	10
Annealing	*T <sub>m1</sub> -5	30 s	
Extension	72	1 min	
Denaturation	94-95	30 s	25
Annealing	**T <sub>m2</sub> -5	30 s	
Extension	72	1 min	
Final Extension	72	10 min	1
Keep	4	∞	

\*T<sub>m1</sub> = Melting temperature considering just the annealing primer region. Theoretically calculated by the IDT tools.

\*\*T<sub>m2</sub> = Melting temperature considering the restrictions sites

4. Load a 5 µL sample and run an agarose gel electrophoresis (**see the section Agarose Gel Electrophoresis and DNA Gel Purification**)  
[[http://2014.igem.org/wiki/images/a/af/Agarose\\_Gel\\_Electrophoresis\\_and\\_DNA\\_Gel\\_Purification.pdf](http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf)].
5. If you see just one band in the gel of the expected size, you can purify the PCR product using Wizard SV Gel and PCR Clean-Up System kit (Promega). But if you see more than one band, you need to run a new gel with total sample, excise the band of interest and then purify (**see the section Agarose Gel Electrophoresis an DNA Gel Purification**)  
[[http://2014.igem.org/wiki/images/a/af/Agarose\\_Gel\\_Electrophoresis\\_and\\_DNA\\_Gel\\_Purification.pdf](http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf)].
6. Use Nanodrop Spectrometer (Thermo Scientific) to quantify the DNA fragment.
7. Store at -20°C.